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# *In Utero* Exposure to Metals and Birth Outcomes in an Artisanal and Small-Scale Gold Mining Birth Cohort in Madre de Dios, Peru

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**Background:** Few birth cohorts in South America evaluate the joint effect of minerals and toxic metals on neonatal health. In Madre de Dios, Peru, mercury exposure is prevalent owing to artisanal gold mining, yet its effect on neonatal health is unknown.

**Objectives:** We aimed to determine whether toxic metals are associated with lower birth weight and shorter gestational age independently of antenatal care and other maternal well-being factors.

**Methods:** Data are from the COhorte de NAcimiento de MAdre de Dios (CONAMAD) birth cohort, which enrolled pregnant women in Madre de Dios prior to their third trimester and obtained maternal and cord blood samples at birth. We use structural equation models (SEMs) to construct latent variables for the maternal metals environment (ME) and the fetal environment (FE) using concentrations of calcium, iron, selenium, zinc, magnesium, mercury, lead, and arsenic measured in maternal and cord blood, respectively. We then assessed the relationship between the latent variables ME and FE, toxic metals, prenatal visits, hypertension, and their effect on gestational age and birth weight.

**Results:** Among 198 mothers successfully enrolled and followed at birth, 29% had blood mercury levels that exceeded the U.S. Centers for Disease Control and Prevention threshold of 5.8 μg/L and 2 mothers surpassed the former 5-μg/dL threshold for blood lead. The current threshold value is 3.5 μg/dL. Minerals and toxic metals loaded onto ME and FE latent variables. ME was associated with FE ($\beta = 0.24$; 95% CI: 0.05, 0.45). FE was associated with longer gestational age ($\beta = 2.31$; 95% CI: −0.3, 4.51) and heavier birth weight. Mercury exposure was not directly associated with health outcomes. A 1% increase in maternal blood lead shortened gestational age by 0.05 d ($\beta = -0.75$; 95% CI: −1.51, −0.13), which at the 5-μg/dL threshold resulted in a loss of 3.6 gestational days and 76.5 g in birth weight for newborns. Prenatal care visits were associated with improved birth outcomes, with a doubling of visits from 6 to 12 associated with 5.5 more gestational days (95% CI: 1.6, 9.4) and 319 g of birth weight (95% CI: 287.6, 350.7).

**Discussion:** Maternal lead, even at low exposures, was associated with shorter gestation and lower birth weight. Studies that focus only on harmful exposures or nutrition may mischaracterize the dynamic maternal ME and FE. SEMs provide a framework to evaluate these complex relationships during pregnancy and reduce overcontrolling that can occur with linear regression. https://doi.org/10.1289/EHP10557

## Introduction

The *in utero* and neonatal periods are critical windows of an individual's life, during which exposures to toxic, metals such as lead (Pb), mercury (Hg), and cadmium (Cd), or a lack of minerals [calcium (Ca), zinc (Zn), iron (Fe), magnesium (Mg)] may have lifelong consequences.[1,2] Many studies have evaluated newborn health outcomes from nutritional or toxicological perspectives, but few have evaluated both simultaneously,[3] especially in developing countries.[4–8] This is of special importance given that nutritional status and toxic exposures are known to interact, causing neonatal health to be dependent on both minerals and toxic metals. Birth cohorts in developed nations benefit from strong health care systems and food security, often precluding the ability to evaluate interactions of malnutrition and toxic metal exposure. A lack of minerals is known to adversely affect fetal development: low levels of Ca can lead to poor skeletal formation and arrythmias in newborns[9]; low levels of Fe during pregnancy increases risk of preterm birth[10,11]; and low levels of Mg are associated with small for gestational age and preterm labor.[12] From a toxicological perspective, maternal exposure to toxic metals during pregnancy is associated with decreased birth weight[5,13–17]; lower ponderal index; shorter gestational age; smaller head circumference; lower appearance, pulse, grimace, activity, and respiration (APGAR) score[18]; and a thinner placenta.[5,19] Maternal Pb exposure has also been found to shorten gestational age.[20] Maternal and fetal Hg exposure has been linked to decreased head circumference[21,22] and cognitive deficits in children.[23,24]

Assessment of exposures to toxic metals and minerals in epidemiological analyses on neonatal health is important because they influence maternal absorption, cellular processes, and nutrient transfer to the fetus.[25–29] For example, Pb and Cd absorption is increased when Fe levels are low. Adequate nutrition could mitigate the harmful effects of toxic metals given that selenium (Se) may sequester harmful free radicals from Hg exposure.[30,31] Toxic metals also compete with minerals for binding sites through molecular mimicry, disrupting cellular functions,[30,32–34] and potentially impeding nutrient transfer to the fetus.[30,33] Cd has been shown to impede the transfer of Zn[26,35] and Ca,[35] and

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increasing levels of maternal Se has been suggested as a means to lower Cd levels in cord blood.[17] The potential for toxic metals to interfere with nutrient transport to the fetus may have important biological consequences that may be undetected when minerals and toxic metals are analyzed separately. Not integrating maternal nutrition in statistical analyses may incorrectly infer the association between toxic metal exposures and neonatal health because effect sizes may be greater in malnourished individuals (or nonexistent in those who are well nourished). This may be of special importance for nutritionally nonreplete populations.

Fetal risk of exposure to minerals and toxic metals exists initially with the maternal environment, or the exposures present during gestation. Minerals, metals, and other nutrients are transferred to the fetus in this environment, representing a physiological hierarchy that is commonly not accounted for in statistical analyses. Birth cohort studies predominantly use standard linear regression to evaluate the direct relationship of either maternal or cord blood measurements to neonatal health. The use of maternal exposures as direct effects on neonatal health and the evaluation of additional variables as effect modifiers does not account for any modifications that may occur in the transfer from mother to child,[4–8,15,19,21,36–43] (Table S1). Although there is a direct association between cord blood and neonatal health, policy and health care recommendations are implemented at the maternal level, requiring an understanding of how the maternal metal environment relates to the fetal environment (FE) and neonatal health. Structural equation models (SEMs) and factor analysis approaches have been proposed to evaluate multivariate exposures and end points[44,45] because they can account for known physiological processes and allow hypothesized structures to be tested statistically. SEMs also permit dependent variables to be evaluated jointly, which prevents overfitting. This issue arises in models evaluating correlated (and endogenous) factors associated with birth weight, such as gestational age, the inclusion of which in linear models limits the amount of remaining variance available to detect the true effects of other variables.[46] SEMs also define unmeasurable (latent) variables that can be constructed and evaluated with newborn health outcomes, such as the maternal metals environment (ME) and the FE. Latent variables can provide a better model fit than linear models.[47] In this study, we used SEMs to test a novel conceptual framework for neonatal health that incorporates the transfer of minerals and toxic metals from mother to child while jointly evaluating gestational age and birth weight.

Few birth cohorts have been implemented in low- and middle-income countries (LMICs). Data collection in LMICs is considerably more difficult compared with similar studies in high-income countries (HICs) for a number of reasons, including resource and infrastructure constraints, building community trust, and local logistical issues. We could not identify any previous birth cohorts that have jointly evaluated nutrients and toxic metal exposures in South America. This is especially important because results from previous birth cohorts in HICs may not be translatable to the Amazon owing to differences in disease burdens, environmental exposures, and health care access.[48] To our knowledge, this is the first birth cohort in South America to evaluate how nutritional status and toxic metal exposures may jointly influence neonatal health outcomes, which are particularly relevant to LMICs currently undergoing nutritional transitions to a Western diet.[49,50] Specifically, we measured direct and indirect effects of toxic metals and minerals to determine their effect on birth weight and gestational age. In doing so, we contribute valuable information in an understudied population on maternal well-being, minerals, and toxic metal exposures. Data are from the COhorte de NAcimiento de MAdre de Dios (CONAMAD) in Madre de Dios, Peru (Figure 1).[51]

## Methods

### *Data Collection*

Data were collected in 2017–2018 as part of the CONAMAD birth cohort based in Madre de Dios, Peru, and was approved by the institutional review board of Universidad Peruana Cayetano Heredia, SIDISI 66471.[51] The cohort is described in detail by Pan et al.[51] Madre de Dios is a hotspot of global biodiversity as well as global Hg pollution owing to the rapid expansion of artisanal and small-scale gold mining (ASGM) over the past two decades. CONAMAD is one of the few birth cohorts conducted in Latin America and the Caribbean that has collected prenatal exposure data and the only birth cohort of which we are aware that is based in an ASGM region. Briefly, 270 mothers were enrolled by health professionals at health posts during a prenatal visit before their third trimester. Enrollment sites were in four zones differentiated by land use and presence of ASGM [Zone 1: ASGM region ($n=37$), Zone 2: ASGM and agriculture ($n=50$), Zone 3: Puerto Maldonado ($n=121$), Zone 4: agriculture ($n=7$)], with no statistically significant differences in birth weight, gestational age, anemia, or hypertension by zone.[51] Inclusion criteria included multiparous women with at least one other child from the same biological father and a planned birth in Madre de Dios because the study was originally designed to evaluate the potential epigenetic effects from Hg exposure. Women were excluded if medically diagnosed with type II diabetes prior to pregnancy, were a current smoker, resided outside Madre de Dios for >2 wk during pregnancy or were planning to give birth outside of Madre de Dios.

At enrollment, study nurses and medical professionals administered a survey on diet, supplements, and transcribed prenatal care records of weight, height, hemoglobin, blood pressure, and prenatal complications. A maternal hair sample was also collected for total Hg determination. At birth, study nurses and medical professionals administered a postnatal survey and collected venous maternal and fetal cord blood samples. Birth weight (in kilograms), gestational age (in weeks), APGAR score, and pathologies of concern were transcribed from medical records onto the prenatal survey.[52] A HemoCue Hb 201+ was used for *in situ* hemoglobin measurements at enrollment and at birth. A total of 215 mothers were successfully followed up at birth. Of those, 1 gave birth to twins, 1 had a stillbirth, and 13 were missing a blood sample and were therefore excluded from the study. Complete data were available for 200 mother–child dyads, consisting of maternal and whole cord blood samples collected in trace element royal blue–topped blood collection tubes. Both blood samples were collected to better understand transplacental transfer of minerals and toxic metals, many of which are bound to red blood cells.[15] The number of prenatal visits was missing for 2 women, resulting in 198 mother–child pairs included in the analysis. Blood samples were initially stored and shipped at −20°C to Duke University (Durham, North Carolina), and subsequently stored at −80°C. All samples were processed in the laboratory of H.H.K.

### *Laboratory Analysis Blood*

Cord and maternal blood samples were analyzed for Pb, Hg, Cd, arsenic (As), Fe, Mg, Zn, sodium, Ca, and Se. The lower limits of quantification and method detection limits are reported in Table S2. All analytes were above detection level, except for Cd (<0.5 μg/L) and As (<0.9 μg/L), which had 68% ($N=135$) and 70% ($N=139$) of samples below the limit of detection (LOD). For samples with concentrations below the lower LOD, a value of half of this limit was assigned to that sample.

For blood digestions, samples were thawed overnight at 4°C, and digested on a hot block at 65°C. The digestions used ultra-trace clean digestion tubes (Environmental Express) and consisted of

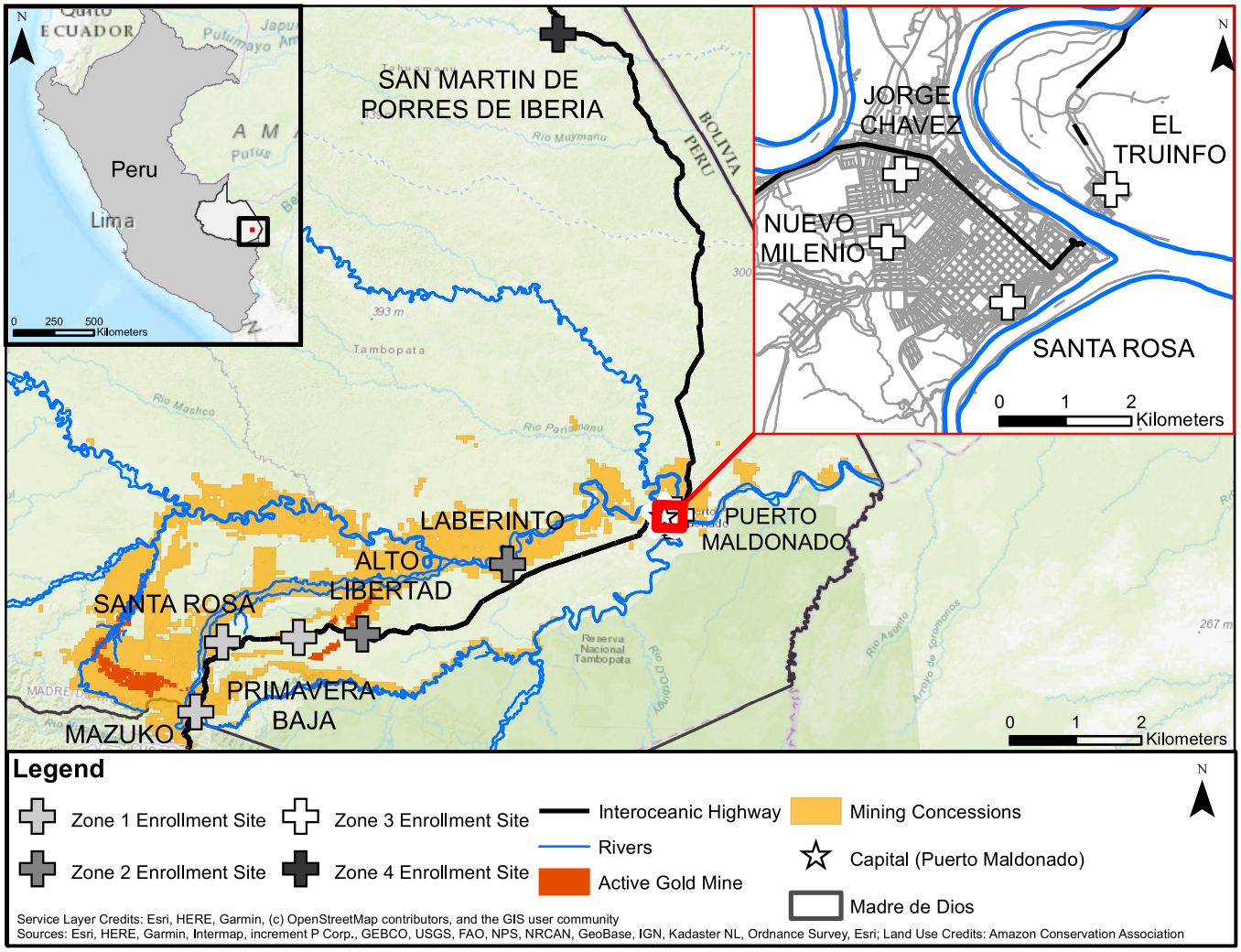


**Figure 1.** CONAMAD Study Region, Madre de Dios, Peru. The capital of Madre de Dios, Puerto Maldonado, is labeled with a star and shown in the red inset map in the upper right. Note: CONAMAD, COhorte de NAcimiento de MAdre de Dios.

heating 0.5 mL of blood with 1 mL of 70% nitric acid ($HNO_3$; Plasma Pure Plus, SCP Science) with 0.05 mL of 30% hydrochloric acid (HCl; Plasma Pure Plus, SCP Science) for 2 h. The samples were cooled and 1 mL of 30% hydrogen peroxide (Plasma Pure Plus, SCP Science) was added to the mixture and heated again for 1 h. After cooling, 10 µL of a 4-mg/L gold (Au)+2% HCl solution was spiked into the digestate to aid in Hg stability. Each digestion batch consisted of 25 blood samples, including two samples analyzed in triplicate. Furthermore, each batch of 25 blood digestions included three blank samples, a National Institute of Standards and Technology (NIST) blood standard reference material (SRM) (levels 2–4), an aqueous standard [High Purity Standards, CRM-TMDW-A + Spex Certiprep (Hg)], and an International Atomic Energy Agency (IAEA) dry blood sample (IAEA-A-13). Analytes were background corrected by subtracting the average of the three blanks. Recoveries for the SRMs are listed in Table S3.

The blood digestates were analyzed by inductively coupled plasma–mass spectrometry (ICP-MS; Agilent 7900). A summary of the plasma conditions is given in Table S2. The samples were diluted (10-fold) into an acid matrix [2% (vol/vol) $HNO_3$ and 0.5% HCl (vol/vol)] consisting of 20 µg/L Au and 20 µg/L internal standards ($^{45}$scandium, $^{89}$yttrium, $^{103}$rhodium, $^{115}$indium, $^{159}$terbium, $^{193}$iridium, and $^{209}$bismuth) to correct for instrumental drift or matrix effects. The analyses were performed in either helium mode (kinetic energy discrimination) or hydrogen mode (reaction mode for $^{78}$Se and $^{44}$Ca). The ICP-MS was optimized to reduce oxide and doubly charged interferences to <3%. The instrument was calibrated for Hg (Brooks Rand), Zn, Pb, Se, and As (Spex Certiprep mix 2A), and Fe, Mg, and Ca (Spex Certiprep custom mix), with the standard curves verified against second source standards [High Purity Standards (CRM-TMDW-A) + Spex Certiprep (Hg)]. Continuing calibration verification checks were performed every 20 samples during a batch run.

### *Laboratory Analysis Hair*

Maternal hair samples were collected at birth by health staff and medical professionals. Methods for hair sample collection and analysis followed Koenigsmark et al.[53] Briefly, hair samples were collected using stainless steel scissors from the occipital region of the head as close to the root as possible. Three samples were collected from each participant and were stored in Ziploc bags with a desiccant. The 2 cm closest to the root were analyzed for total Hg using a Direct Mercury Analyzer-80 (DMA), representing the last 2 months of exposure.[54] Dilutions of certified 1 mg/L total Hg standard (Brooks Applied Labs) were used to calibrate the instrument. A certified reference material (CRM DB001; European Reference Materials) was processed to assess the DMA calibration over the course of the hair analysis. The CRM recovery was 96%

($n = 79$). For a subset of samples ($n = 49$), the CRM, IAEA-086, was used and had a recovery of 92% ($n = 8$).

### Variables

***Outcomes.*** The study outcomes were gestational age (in weeks) and birth weight (in grams). Both were normally distributed. Gestational age was determined by the Capurro somatic method.[52] Birth weight was measured at birth by medical staff. Only singleton births were included for analysis.

***Predictors.*** Minerals (Zn, Mg, Ca, Se, Fe) and toxic metals (Pb, Hg, As, Cd) measured in maternal and cord blood were the tested predictors of gestational age and birth weight. Analyte measurements were normalized with a $\log_{10}$ transformation. Two latent variables, maternal ME and FE, were constructed from measured minerals and toxic metal concentrations in maternal and cord blood, respectively. The ME and FE environments were created by loading minerals and toxic metals measured in maternal and cord blood, respectively. Loadings that had an absolute value of <0.30 were removed from the model.

***Covariates.*** Covariates include survey data consisting of mother's age, whether the mother took nutritional supplements during pregnancy (yes/no), number of previous births, and socioeconomic status (SES). Nutritional supplements included folic acid, Fe tablets, and iron sulfate ($FeSO_4$) taken for various durations and doses prescribed by health officials. The variable "number of births" had a nonnormal distribution (median = 2, range: 1–7) and was evaluated as a binary variable categorized as 1–3 births ($n = 166$) and ≥4 births ($n = 16$). We also evaluated SES, which was determined using estimated household annual income from the partner's occupation and classified as below minimum wage [<12,000 Peruvian Nuevo Soles (PNS)], low (10,200–14,000 PNS), and moderate (>14,000 PNS), with 1 PNS = USD $0.295 (November 2016). It is important to note that Peru set the minimum wage in 2016 at 850 PNS/month (or 10,200 PNS annually), indicating that incomes in Madre de Dios were low compared with national standards.

At each prenatal care visit, mothers were screened for anemia (using hemoglobin measurements), gestational hypertension, and gestational diabetes by medical professionals. The mean number of prenatal visits was 7 and ranged from 2 to 12 visits (Table 1). Whether or not a mother developed gestational diabetes (yes/no), hypertension (systolic blood pressure >140 mmHg, yes/no), or pathologies of concern (risk of abortion, previous cesarian delivery, urinary infection, bleeding, and hyperemesis gravidarum) was transcribed from medical records. Hemoglobin measurements at enrollment and at birth, as well as presentation of anemia during pregnancy, were also assessed. Hemoglobin was measured using a HemoCue Hb 201+, with anemia classification dependent on the estimated gestational week.[55] The number of prenatal care visits was confirmed with the clinical record. The data set did not include maternal body mass index prior to pregnancy. Total Hg in maternal hair at birth was also evaluated as a risk factor for neonatal health by incorporating maternal hair Hg into the SEM. The potential for outcomes to be modified by newborn sex was also assessed.

### Statistical Analysis

***Conceptual framework.*** Minerals and toxic metals are known to interact at multiple physiological levels from absorption rates to distribution within the body. To account for potential interactions between minerals and toxic metals, we created latent variables that represented the maternal ME and the FE. We then statistically evaluated how the ME was associated with the FE and their associations with birth weight and gestational age while accounting for covariates (age, sex, hypertension, and number of prenatal care visits; Figure 2).

**Table 1.** Characteristics of maternal and newborn health in the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018 ($n = 198$).

| | Overall ($N = 198$) |
|---|---|
| Mother's age (y) | |
| Mean ± SD | 27.5 ± 4.16 |
| Median (min, max) | 27.0 (18.0, 35.0) |
| Hypertension [*n* (%)] | |
| No | 178 (93.7) |
| Yes | 12 (6.3) |
| Missing | 8 |
| Diabetes status [*n* (%)] | |
| No | 196 (99.5) |
| Yes | 1 (0.5) |
| Missing | 1 |
| Pathologies of concern [*n* (%)] | |
| None | 163 (90.6) |
| Previous cesarean section | 3 (1.7) |
| Abortion risk | 1 (0.6) |
| Hyperemesis gravidarum | 1 (0.6) |
| Urinary tract infection | 1 (0.6) |
| Placenta previa | 1 (0.6) |
| Bleeding and pain | 1 (0.6) |
| Unspecified | 9 (5.0) |
| Missing | 18 |
| Socioeconomic status [*n* (%)] | |
| Low | 31 (17.2) |
| High | 63 (35.0) |
| Moderate | 86 (47.8) |
| Missing | 18 |
| Number of previous births | |
| Mean ± SD | 1.95 ± 1.09 |
| Median (min, max) | 2.00 (1.00, 7.00) |
| Missing | 16 |
| Prenatal care visits | |
| Mean ± SD | 7.25 ± 2.12 |
| Median (min, max) | 7.00 (2.00, 12.0) |
| Maternal hemoglobin at enrollment (g/dL) | |
| Mean ± SD | 11.7 ± 1.25 |
| Median (min, max) | 11.6 (7.00, 15.7) |
| Missing | 8 |
| Maternal hemoglobin at birth (g/dL) | |
| Mean ± SD | 11.4 ± 1.08 |
| Median (min, max) | 11.3 (8.70, 14.8) |
| Missing | 32 |
| Presented anemia during pregnancy [*n* (%)] | |
| No | 129 (80.1) |
| Yes | 32 (19.9) |
| Missing | 37 |
| Trimester of anemia presentation [*n* (%)][a] | |
| Anemic first trimester | 1 (0.6) |
| Anemic second trimester | 1 (0.6) |
| Anemic at birth | 30 (18.6) |
| Not anemic at first trimester | 40 (24.8) |
| Not anemic at second trimester | 64 (39.8) |
| Not anemic at birth | 25 (15.5) |
| Missing | 37 |
| Newborn's sex [*n* (%)] | |
| Female | 87 (43.9) |
| Male | 111 (56.1) |
| Birth weight (g) | |
| Mean ± SD | 3,540 ± 454 |
| Median (min, max) | 3,560 (1,650, 5,050) |
| Gestational age (wk) | |
| Mean ± SD | 39.2 ± 1.22 |
| Median (min, max) | 39.0 (33.0, 42.0) |
| Preterm pregnancy [*n* (%)][b] | |
| No | 191 (96.5) |
| Yes | 7 (3.5) |

Note: CDC, Centers for Disease Control and Prevention; CONAMAD, COhorte de NAcimiento de MAdre de Dios; max, maximum; min, minimum; SD, standard deviation.
[a]Anemia was determined by gestational age as described by the CDC.[55]
[b]Preterm was classified as a pregnancy <37 wk.

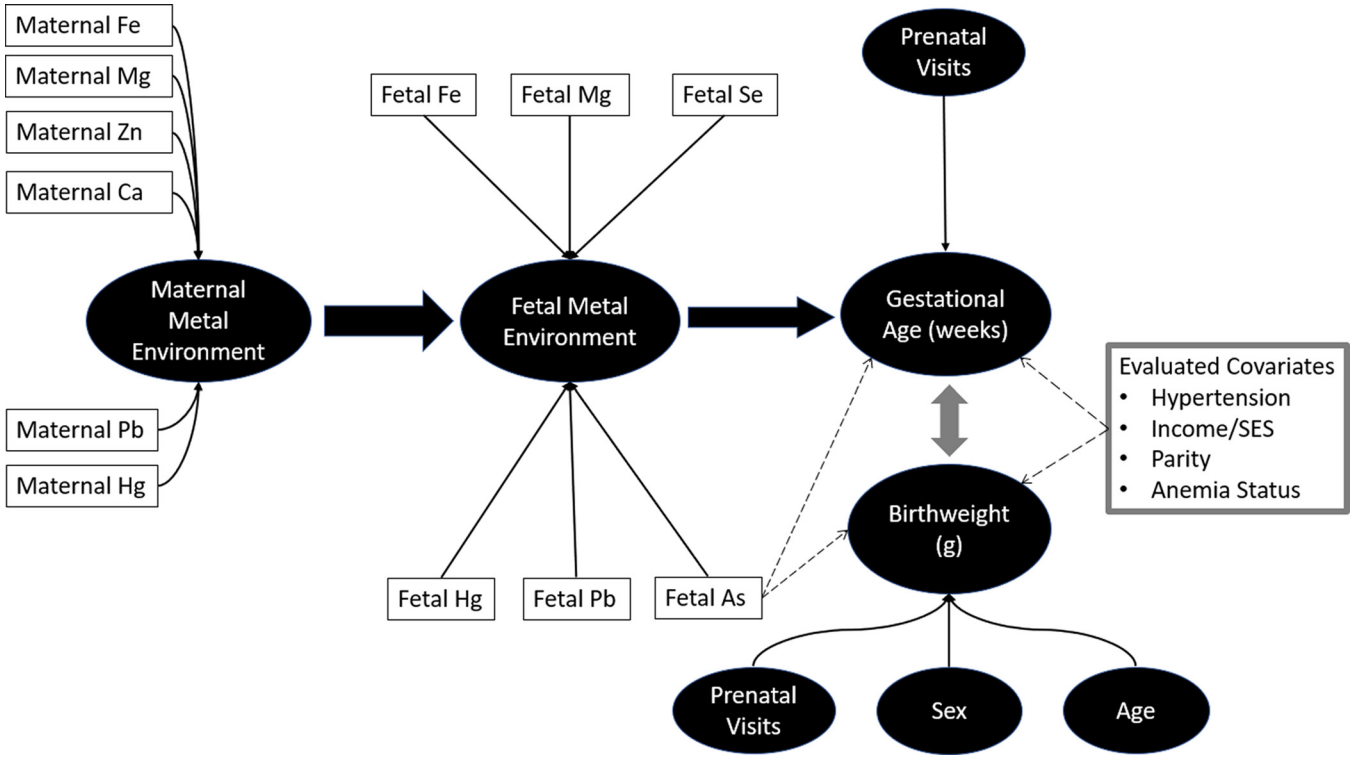


**Figure 2.** Theoretical latent model with evaluated covariates. Note: anemia status (yes/no), measured hemoglobin values below recommended guidelines by gestational week at enrollment and birth; As, arsenic; Ca, calcium; Fe, iron; Hg, mercury; hypertension, the development of gestational hypertension (systolic blood pressure >140 mmHg); Mg, magnesium; parity, number of prior births; Pb, lead; prenatal visits, number of prenatal care visits prior to birth; Se, selenium; SES, socioeconomic status; Zn, zinc.

***Measurement model.*** To incorporate the transport of minerals and toxic metals from the mother to the fetus, latent variables reflecting the ME and the FE were created from maternal blood at birth and cord blood, respectively. ME and FE comprised minerals and toxic metals that loaded onto the latent variable, using a $p < 0.05$. Preliminary models, which evaluated the loading of maternal blood and cord blood onto their respective latent variables, without adjustments, demonstrated an association between ME and FE (Table S4).

***Model identification.*** SEMs were used to test the causal structure of the physiological hierarchy, with the ME indirectly affecting gestational age and birth weight through the FE (Figure 2). We then individually evaluated the direct effect of risk factors (hypertension, diabetes, anemia, SES, and parity) and toxic metals on gestational age and birth weight; given that birth weight is dependent on gestational age, we evaluated them jointly.

Age was evaluated continuously and as a binary variable, in separate models, to compare the top and bottom 50th percentiles. We found no nonlinear associations with a continuous maternal age variable and used this for subsequent models (Table S5). Maternal hemoglobin values at enrollment and at birth, as well as presentation of anemia during pregnancy (yes/no) and maternal hair Hg concentration at birth, were also evaluated. The direct effect of the ME, toxic metals, and minerals on gestational age and birth weight was also assessed. We used Pearson correlation statistics to evaluate the correlations of toxic metals and minerals within and between the maternal and cord blood.

Model estimates were quantified using maximum likelihood and evaluated using chi squared ($\chi^2 > 0.05$), Tucker-Lewis Index (TLI $> 0.95$), root mean square error of approximation (RMSEA $< 0.07$) and standardized root mean residual (SRMR $< 0.08$). Models that met these requirements were further evaluated and compared using the Akaike information criterion (AIC) and the Bayesian information criterion (BIC), with the lowest values demonstrating the best fit model (Table 2). Analysis was done in RStudio (version 1.4.1717). Model fitting involved the selection of a base model that adjusted for common risk factors (hypertension, sex, age, and number of prenatal visits), and then the addition of Pb and Hg to the base model. Only mother–child pairs with complete data were included in the analysis. Base model results with Pb or Hg were identical to the base model, thus, only the final model results are shown here. A threshold of 0.05 was used for statistical significance.

## Results

The study analyzed 198 mother–child pairs of singleton births. Most women presented no pathologies of concern (82%) and had full-term pregnancies (96%). Only 6% of women became hypertensive (systolic blood pressure >140 mmHg or diastolic >90 mmHg)

**Table 2.** Global fit measures of base and maternal Pb SEMs.

| Model | df | $\chi^2$ ($p$-value) | CFI/TLI | RMSEA (CI) | SRMR | AIC | BIC |
|---|---|---|---|---|---|---|---|
| Base model | 116 | 0.08 | 0.985/0.981 | 0.031 (0.000, 0.049) | 0.074 | −3,314.6 | −3,166.6 |
| Maternal Pb | 116 | 0.06 | 0.984/0.980 | 0.032 (0.000, 0.050) | 0.073 | −3,311.6 | −3,163.5 |

Note: AIC, Akaike information criterion; BIC, Bayesian information criterion; CFI/TLL, comparative fit index/Tucker-Lewis index; CI, confidence interval; df, degrees of freedom; Pb, lead; RMSEA, root mean square error of approximation; SEMs, structural equation models; SRMR, standardized root mean squared residual.

and 0.5% were diagnosed with diabetes during pregnancy. Women had an average of 7.3 prenatal visits, 80% took some type of nutritional supplement, and most were pregnant with their second child (Table 2). Most women had moderate and higher household incomes (43% and 32%, respectively). Less than a third (28.8%) of mothers had blood Hg levels that surpassed the U.S. Centers for Disease Control and Prevention (CDC) guidelines of 5.8 μg/L.[56] Total Hg in maternal hair ranged from 0.2 μg/g to 8.4 μg/g, with a mean of 1.9 μg/g. Two mothers surpassed the CDC's former 5 μg/dL limit for total blood Pb (Table 3).[57] Seven mothers surpassed the CDC's current limit, passed on October 2021, for total blood Pb of 3.5 μg/dL.

We found correlations ($p < 0.05$) among toxic metals and minerals within maternal blood and cord blood, as well as between maternal blood and cord blood (Figure 3; Figure S1). Overall, metals grouped by sample type (maternal or fetal), with the notable exceptions of Hg, Pb, Cd, and Mg, which were not part of a larger correlated group (Figure S1). Hg and Pb were not correlated with other metals.

### *Relationship of Variables with the Maternal ME*

In maternal blood, Fe was positively correlated with Se, Zn, and Mg. Ca was inversely correlated with Zn and Fe (Figure 3). Levels of Ca, Se, Hg, and Pb were positively correlated between maternal and fetal blood, with Pb and Hg having the strongest correlations [0.90 and 0.80, respectively (Figure 3; Table S6)].

In establishing the ME, mineral concentrations had the greatest influence on the ME (largest loading factors), with toxic metals having a lesser contribution. Fe, Zn, Pb, Hg, Mg, and Ca contributed to the latent variable ME, with loading values of 1.25, 1.24, 1.22, 1, 0.46, and −0.30, respectively. ME values ranged from −0.21 to 0.20. All toxic metals and minerals were positively associated with ME, except for Ca. Cd did not load onto ME.

**Table 3.** Minerals and toxic metals measured in maternal and cord blood at birth for the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018.

| | Cord blood[a] ($N = 198$) | Venous blood[a] ($N = 198$) | $p$-Value[b] |
|---|---|---|---|
| Hg (μg/L) | | | |
| GM ± SD | 6.0 ± 2.4 | 3.6 ± 2.6 | 0.001 |
| 95% CI | 1.5, 25.6 | 0.79, 16.5 | — |
| Pb (μg/dL) | | | |
| GM ± SD | 1.1 ± 1.8 | 1.4 ± 1.7 | 0.006 |
| 95% CI | 0.46, 2.88 | 0.67, 3.2 | — |
| Cd (μg/L) | | | |
| GM ± SD | 0.24 ± 1.7 | 0.32 ± 1.7 | 0.008 |
| 95% CI | 42.4, 86.1 | 51.0, 83.2 | — |
| As (μg/L) | | | |
| GM ± SD | 0.61 ± 1.7 | 0.64 ± 1.7 | 0.442 |
| 95% CI | 0.5, 2.1 | 0.5, 2.2 | — |
| Fe (mg/L) | | | |
| GM ± SD | 524.4 ± 1.2 | 378.4 ± 1.3 | <0.001 |
| 95% CI | 372.2 | 687.9 | — |
| Ca (mg/L) | | | |
| GM ± SD | 60.3 ± 1.2 | 65.9 ± 1.2 | <0.001 |
| 95% CI | 42.4 | 86.1 | — |
| Mg (mg/L) | | | |
| GM ± SD | 34.8 ± 1.1 | 33.8 ± 1.1 | 0.03 |
| 95% CI | 28.8 | 44.5 | — |
| Zn (mg/L) | | | |
| GM ± SD | 1.9 ± 1.4 | 4.9 ± 1.3 | <0.001 |
| 95% CI | 1.3, 3.2 | 3.1, 7.4 | — |
| Se (μg/L) | | | |
| GM ± SD | 162.0 ± 1.2 | 157.1 ± 1.2 | 0.15 |
| 95% CI | 117.9, 226.5 | 109.4, 223.5 | — |

Note: —, not applicable; As, arsenic; Ca, calcium; Cd, cadmium; CI, confidence interval; CONAMAD, COhorte de NAcimiento de MAdre de Dios; Fe, iron; GM, geometric mean; Hg, mercury; LOD, limit of detection; Mg, magnesium; Pb, lead; SD, standard deviation; Se, selenium; Zn, zinc.

[a]GM and 95% CI values of toxic metals and minerals in blood. Values <LOD were imputed as half of the LOD.

[b]Fisher's $T$-test between maternal blood and cord blood for each toxic metal and mineral.

### *Relationship of Variables with the FE*

Within cord blood, Cd was correlated with Zn and Ca, and inversely correlated with Fe and Se. Fe was correlated with Se and inversely associated with Ca.

A different set of toxic metals and minerals was found to load onto FE and comprised Hg, Fe, As, Se, Pb, and Mg, with loading values of 1.0, 0.96, 0.93, 0.71, 0.53, and 0.36. FE values ranged from −0.22 to 0.19. Cd and Ca did not load onto FE.

### *Relationship between Maternal ME, FE, and Neonatal Outcomes*

***Gestational age.*** The latent variable ME was associated with FE in both the base and final SEMs (Table S7 and Figure S2). After adjusting for hypertension, age, sex, prenatal visits, and maternal blood Pb, the FE had a positive effect on birth outcomes given that it was associated with gestational age [β = 2.28; 95% confidence interval (CI): 0.02, 4.63], with an additional unit increase in the FE lengthening gestational age by 2 wk. Hypertension was associated with a shortened gestational age of 4 d (β = −0.60; 95% CI: −1.25, 0.04; Table 4, Figure 4). The number of prenatal care visits was associated with longer gestation (β = 0.13; 95% CI: 0.05, 0.23), with each prenatal care visit adding almost an extra day of gestation (Figures S3 and S4 and Table S8).

A 1% increase in maternal Pb was associated with a shorter gestational age of 0.05 d [β = −0.74; 95% CI: −1.46, −0.16 (Table 4, Figure 4)], which at the 5-μg/dL threshold, resulted in a loss of 3.6 gestational days (95% CI: 0.6, 7.4) and 76.5 g (95% CI: 13.6, 139.4) birth weight for newborns. Compared with the base model, the inclusion of maternal blood Pb level did not modify the effect of hypertension status (Table 4), potentially indicating independent mechanisms through which Pb and hypertension affect gestational age. Mothers with hypertension and average blood Pb levels had a shorter gestational age of 5.3 days (95% CI: 0.22, 11.05). Similar results were found even when excluding women with outlying Pb exposure. According to the standardized SEM solution, a 1-standard deviation (SD) increase in the FE score has an equivalent and opposite effect to a 1-SD increase in the $\log_{10}$ maternal Pb exposure, indicating that a positive increase in FE may counterbalance the negative association of maternal Pb exposure on neonatal birth outcomes. For example, mothers who had an FE score in the 75th percentile could have 34% higher blood Pb levels before a negative loss in gestational days compared with mothers with an FE at the 25th percentile (Figure 5, Table 4). Maternal hair Hg concentrations at birth were not associated with birth outcomes (Table S9). Maternal hemoglobin concentrations at enrollment and at birth and anemia status during pregnancy were also not associated with birth outcomes (Tables S10–S12).

***Birth weight.*** Birth weight was associated with mother's age in years, (β = 18.43; 95% CI: 5.99, 31.67), number of prenatal visits (β = 51.3; 95% CI: 20.78, 85.40), and newborn's sex, with females weighing 127 g less than males (β = −127.43; 95% CI: −242.48, −12.84). For each additional year of age, a mother's child weighed an additional 18.4 g. Each prenatal visit was associated with an increase of 51.3 g (95% CI: 20.78, 85.40), with a doubling of visits from 6 to 12 associated with 5.5 more gestational d (95% CI: 2.1, 9.7) and 307 g of birth weight (95% CI: 124.68, 512.4). The updated World Health Organization (WHO)

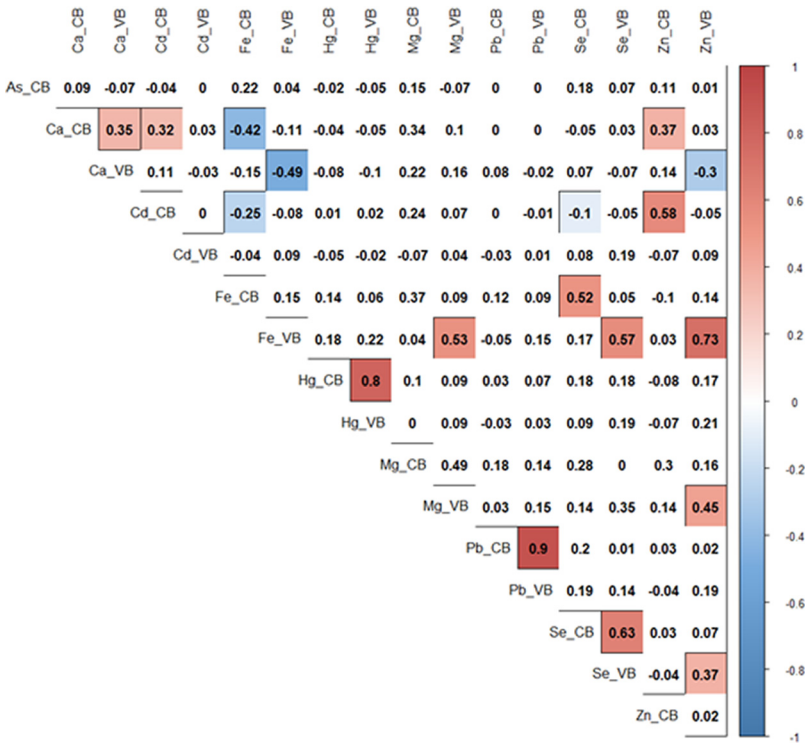


**Figure 3.** Heat map of minerals and toxic metal correlations in maternal (VB) and cord (CB) blood for the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018. The Pearson correlation value is shown for maternal and fetal minerals/toxic metals, with significant positive and negative correlations represented by red and blue shading, respectively ($p < 0.05$). Nonsignificant correlations are white. *p*-Values are provided in Table S6. Note: As, arsenic; Ca, calcium; Cd, cadmium; CONAMAD, COhorte de NAcimiento de MAdre de Dios; Fe, iron; Hg, mercury; Mg, magnesium; Pb, lead; Se, selenium; Zn, zinc.

guidelines of increasing prenatal health visits from 4 to 8 was associated with a 205.2-g (95% CI: 83.12, 341.6) increase in birth weight.[58]

Although Hg loaded on the latent variables ME and FE, Hg in maternal or cord blood was not directly associated with gestational age or birth weight. Nor did we find a direct relationship between ME and gestational age or birth weight. Maternal hair Hg at birth, hemoglobin levels at enrollment, hemoglobin levels at birth, and anemia status during pregnancy were also not associated with birth outcomes (Tables S9 and S12). Last, we did find a covariance between birth weight and gestational age ($\beta = 146.0$; 95% CI: 41.0, 280.1), with birth weight increasing 146.0 g for each additional gestational week, providing further support to analyze these outcomes jointly (Table 4).

## Discussion

In this study, we used SEMs to integrate the physiological hierarchy present during gestation and evaluate the joint effect of toxic metals and minerals on neonatal health. We find that maternal Pb exposure below the former CDC threshold of 5 μg/dL is associated with shorter gestational age and lower birth weight. This

**Table 4.** Final structural equation model predicting gestational age and birth weight for the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018.

| | β (95% CI)[a] | *p*-Value | Std. lv | Std. all |
|---|---|---|---|---|
| Base model with maternal Pb | | | | |
| Fetal ME[b] | | | | |
| ME | 0.24 (0.05, 0.45) | 0.03 | 0.22 | 0.22 |
| Gestational age (wk)[b] | | | | |
| FE | 2.28 (0.02, 4.63) | 0.05 | 0.18 | 0.15 |
| Prenatal visits (*n*) | 0.13 (0.05, 0.23) | 0.004 | 0.13 | 0.23 |
| Hypertension (Ref: no) | −0.60 (−1.25, 0.04) | 0.06 | −0.60 | −0.12 |
| $Log_{10}$ maternal Pb (μg/dL) | −0.74 (−1.46, −0.16) | 0.03 | −0.74 | −0.14 |
| Birth weight (g)[b] | | | | |
| Prenatal visits (*n*) | 51.3 (20.78, 85.40) | 0.001 | 51.3 | 0.24 |
| Mother's age (y) | 18.43 (5.99, 31.67) | 0.002 | 18.4 | 0.17 |
| Sex (Ref: male) | −127.43 (−242.48, −12.84) | 0.03 | −127.4 | −0.14 |
| Raw covariance structure | | | | |
| Gestational age ~ birth weight[c] | 146.04 (41.01, 280.10) | 0.02 | 146.04 | 0.30 |

Note: The model adjusts for hypertension, age, sex, and maternal blood Pb with latent variables for the maternal ME and the FE ($n = 198$). Latent variables ME and FE are unitless. Gestational age and birth weight are evaluated simultaneously with the model structure shown in Figure 4. The ~ symbol represents the covariance between gestational age and birth weight. CI, confidence interval; CONAMAD, COhorte de NAcimiento de MAdre de Dios; FE, fetal environment; g, grams; ME, metals environment; n, number of prenatal care visits; Pb, lead; Ref, reference; Std. all, standardized all (completely standardized solution); Std. lv, standardized latent variable (β coefficient only standardizing the latent variables); wk, week; y, yes.
[a]Raw coefficients.
[b]Health outcomes.
[c]Evaluated covariates.

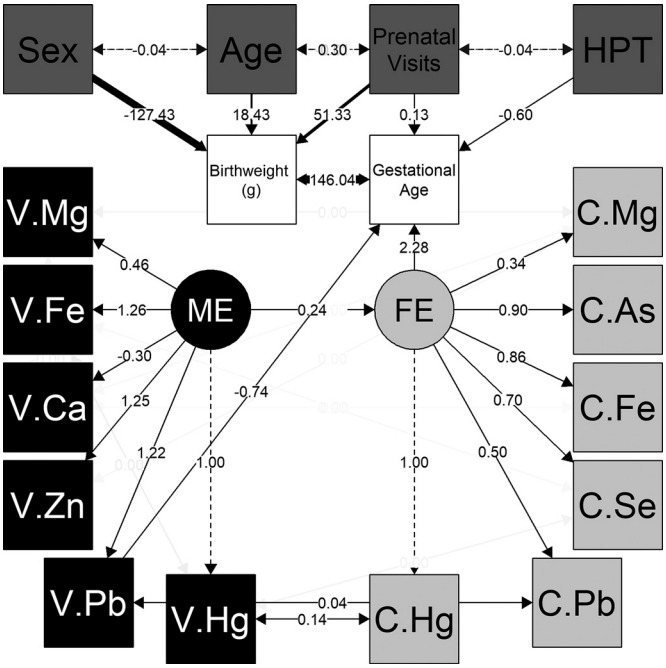


**Figure 4.** Final model diagram of toxic metals, minerals, and covariates on birth outcomes of concern for the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018. Final model diagram includes hypertension (HPT), age, sex, prenatal visits, and maternal blood Pb levels (μg/dL), with the maternal metals environment and fetal metals environment as latent variables (ME and FE, respectively). Values shown are unstandardized β values with transparency dependent on *p*-value as provided in Table 4. Boxes are colored by maternal blood (black), cord blood (light gray), traditional covariates (dark gray), ME (black), FE (light gray), and neonatal health outcomes (white). Note: As, arsenic; Ca, calcium; CONAMAD, COhorte de NAcimiento de MAdre de Dios; Fe, iron; Hg, mercury; Mg, magnesium; Pb, lead; Se, selenium; Zn, zinc.

finding was dependent on measures of the maternal ME, the FE, and other known determinants of neonatal health outcomes that were evaluated within a realistic physiological hierarchy. This finding supports the idea that even low levels of Pb exposure can have important consequences for fetal health. Unexpectedly, maternal Hg levels were not directly associated with either birth outcome, although Hg levels did load into the maternal ME and FE. The specification of the latent variables maternal ME and FE is unique in toxicological and epidemiological approaches and offers an important tool for evaluating joint effects of nutritional and toxic metal exposure levels. Our data suggest that minerals are an important determinant of the maternal ME and the FE, with toxic metals having a lesser contribution, as shown by loading factors. Using this physiologically based approach, we found that maternal ME is associated with the FE, which, in turn, may mediate the effects of stressors such as Pb and maternal hypertension.

An important finding from this study is the beneficial effect of prenatal care on neonatal health. We found that an increase in prenatal care visits from 6 to 12 is associated with an increase gestation by 5.5 d and increase birth weight by 320 grams. Although Peru recommends 6–8 prenatal care visits, the number of visits in the CONAMAD cohort ranged from 2 to 12.[59] Guidelines for prenatal care vary across countries. The United States recommends 12 prenatal care visits,[60] whereas Japan[61] and the WHO[58] recommend 14 and 8 prenatal care visits, respectively, for low-risk pregnancies. Although age, sex, and hypertension are known covariates for neonatal health, the number of prenatal care visits is often underappreciated in epidemiological studies,[4–8,15,19,21,36–43] (Table S1), regardless of the well-found benefits of prenatal care, which include prescription of nutritional supplements, maternal and fetal assessment, and increased knowledge on strategies to alleviate physiological symptoms.[58] In Peru, prenatal care visits are free, covered by the national health care system, reducing the possibility that the number of prenatal care visits is a proxy for wealth. However, the CONAMAD study demonstrated that there remains considerable variability in access and utilization of prenatal care, which is a potential area of focus as Madre de Dios and Peru overall seek to improve neonatal health.

This study also revealed numerous metal and mineral correlations within and across maternal and cord blood, demonstrating their interconnected nature. This is due to differences in their ability to access metal transporters, dependent on molecular structure, compatibility, and competing metals. Mechanistic details on transport, although not explained in detail here, can be found in the literature.[62–65] In birth cohort studies, this complexity poses a methodological limitation when toxic metals or minerals are evaluated individually because it omits important changes occurring in other analytes that may be associated with health outcomes. A prime example of this interaction is the role of Fe levels on Pb exposure given that individuals with low Fe are known to absorb greater amounts of Pb than Fe-replete individuals.[57] Minerals are also known to interact with each other.[66,67] Previous epidemiological studies found negative correlations between serum ferratin and Ca[68] and Ca supplementation was found to decrease heme and nonheme Fe absorption in humans[67] and rats.[69] Although Ca is expected to not limit Fe absorption in populations that consume a Western diet, less is known for those who eat a non-Western diet and for pregnant women.[70] Maternal Ca levels in the CONAMAD cohort were lower than in HICs, where 86.4–92 mg/L Ca were measured at birth, compared with 66.6 mg/L.[71–73] We found maternal Ca loaded negatively onto ME and may reflect the relationship between Fe and Ca. The negative effect of Ca on ME, may provide evidence that Ca is limiting Fe absorption and that Ca supplementation should not be taken with main meals so as to limit any suppression of Fe absorption.[74] Given this complexity, the single toxic metal–single health outcome paradigm may not be appropriate. To better understand maternal and neonatal health outcomes, the effects of minerals and toxic metals and their potential interactions need to be further evaluated.

This is one of the first birth cohorts in Peru and the Amazon to focus on minerals, toxic metals, prenatal care, and neonatal health. It is also unique in that it was conducted in a region where artisanal gold mining is prevalent, and women live in relatively rural, remote areas. Compared with birth cohorts in HICs, CONAMAD had lower levels of Zn[75–77] but its levels were similar to those of other middle-income countries[78] and were not associated with adverse health outcomes.[79] We also found lower levels of Se, Cd, and Pb, but much higher levels of Hg, in our population compared with the Boston Birth Cohort.[42] Cd levels in CONAMAD are lower than other studies that found adverse neonatal health effects.[80–82] Although we did not find any correlations between maternal Cd and maternal Zn or Se less than $p < 0.05$, previous studies in HICs found an inverse correlation between Se and Cd.[83] Interestingly, Se and Cd were positively correlated in birth cohorts in low-income communities in the United States,[84] in smoking populations in Eastern Europe,[85] and in healthy Japanese women,[82] exemplifying the importance of studying populations with different diets and confounding factors.

This is also one of the first birth cohorts studying *in utero* metal concentrations using SEMs[46,86] to model their transfer from mother to child and jointly determine their associations with gestational age and birth weight while accounting for antenatal care and other confounding factors. By creating latent variables for ME and FE, we demonstrated the feasibility and utility of SEMs to evaluate metal mixtures and their effect on neonatal health. Compared with

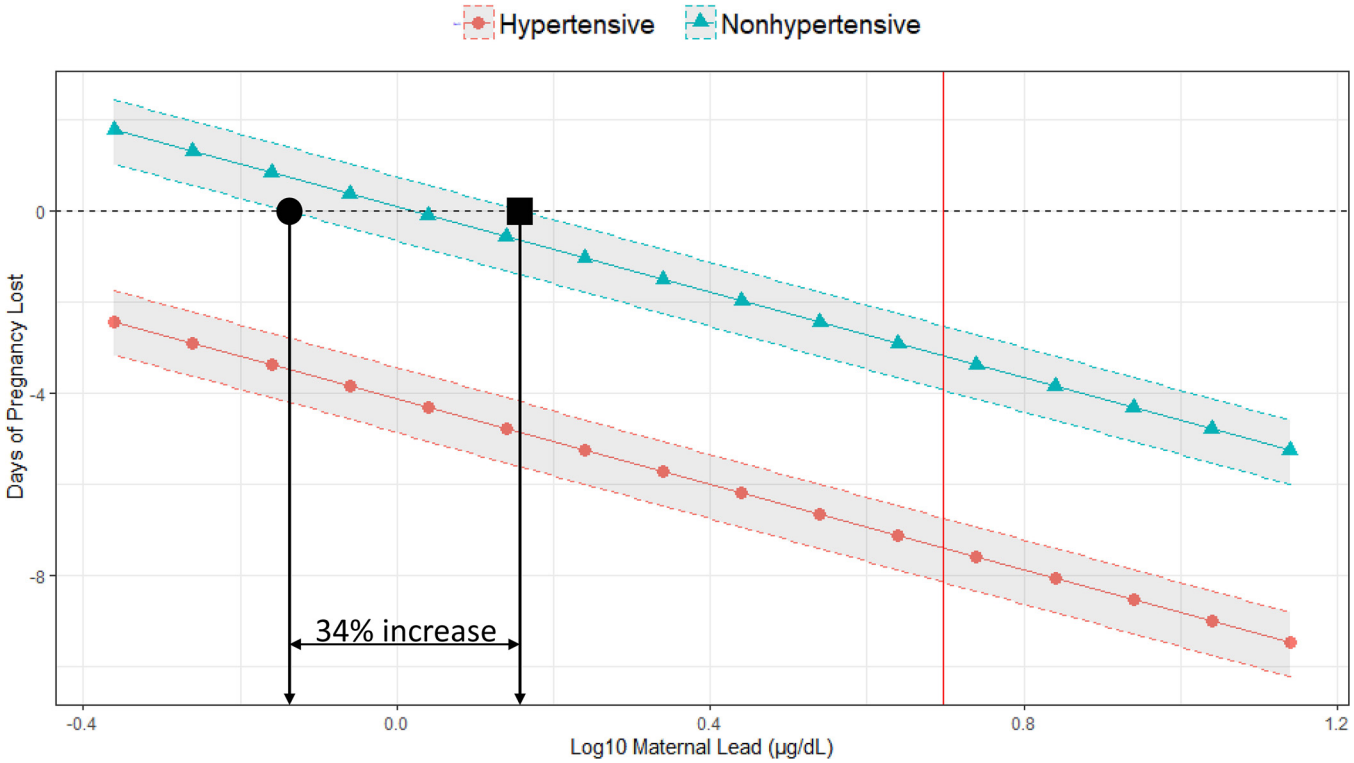


**Figure 5.** Effect of maternal Pb levels and hypertension on gestation for the CONAMAD birth cohort conducted in Madre de Dios, Peru, from 2017 to 2018. Days of pregnancy lost from $\log_{10}$ Pb maternal blood concentration (µg/dL) for mothers with an average FE (50th percentile, center, dotted lines) with the 25th and 75th quartiles for FE (shaded region and dashed lines). Horizontal, black dashed line represents no days of pregnancy lost. Hypertensive and nonhypertensive mothers are shown as red circles and blue triangles, respectively. Values used are from Table 4. The circle and square demonstrate where days of pregnancy lost become negative for the 25th and 75th percentiles of the FE, respectively, demonstrating a 34% increase in Pb exposure before the 75th percentile suffers days of lost pregnancy. Vertical red line represents the former 5-µg/dL reference level set by the U.S. Centers for Disease Control and Prevention. Note: FE, fetal environment; Pb, lead.

linear regression, SEMs allow for a more complete understanding of the structural associations present in maternal–fetal health by assessing direct and indirect effects, limiting overcontrolling, and allow outcomes to be modeled jointly to evaluate the entire physiological system.[46] These characteristics make SEMs a useful analytical tool in evaluating the causal links that determine health outcomes. There has been recent support for the development of new statistical methods for analyzing complex mixtures that move beyond traditional regression.[87] SEMs provide a valuable compliment to better understanding mixtures with their ability to create latent variables, evaluate joint outcomes, and test hierarchical models. In contrast to dose–response models, where a single toxin or exposure is evaluated against a single health outcome, the SEM allows us to integrate multiple toxins, minerals, or other nutrients within a hierarchical physiological framework. Some studies have discussed the trade-offs between SEMs and traditional dose–response models in toxicological risk assessments.[44,88,89]

Although Hg loaded onto ME and FE, we did not find a direct association between maternal or cord blood Hg levels with evaluated birth outcomes. We also evaluated maternal total hair Hg at birth, reflecting the last 2 months of exposure, which was also not associated with health outcomes (Table S9). The lack of a direct Hg exposure effect may be due to the exposure assessment (cord and maternal blood) being related to exposure time periods around birth rather than during conception or other earlier periods of fetal growth. It is possible that a segmented analysis of maternal hair, which approximates methylmercury (MeHg) exposure during different periods of the pregnancy, would have been a more appropriate biomarker of exposure. Other possible reasons for the lack of effect are the beneficial effects of polyunsaturated fatty acids from fish consumption[90] and the high prevalence of fruits with elevated levels of antioxidants in the diet.[91,92] Hg exposures in the region are also predominantly MeHg, targeting the central nervous system and may not be captured by gestational age or birth weight.

This study has several important limitations. First, as noted above, the blood metal concentrations reflect only those at the time of birth, and we do not account for maternal or *in utero* exposures to minerals or toxic metals during the first and second trimester. Although we did obtain data on nutritional supplements and hemoglobin levels at two time periods during pregnancy, both were unrelated to birth outcomes (Tables S10–S12). This is important because the critical window of exposure may occur early in pregnancy, depending on the mineral/metal of concern. Maternal blood Pb levels follow a U-shaped curve as they decline early in pregnancy from plasma volume expansion and increase until delivery owing to increased absorption and mobilization of Pb stored in bone, coinciding with increased Ca demand for the fetus in the third trimester.[20,93,94] In agreement with our findings, Rabito et al. found third trimester blood Pb levels associated with gestational age and not weight. However, they did find second and third trimester blood Pb levels to be associated with preterm birth.[20] There is also evidence that the first trimester maternal Pb levels are more strongly associated with adverse cognitive outcomes in children than the third trimester; however, both were associated with child cognition.[95] We also were not able to account for any additional adverse exposures, such as air pollution, inorganic chemicals, pesticides, or household chemicals. There was also a potential bias in requiring two children from the same biological father given that, anecdotally, local health professionals identified such families as being uncommon. This condition may have resulted in finding

less exposure risk to Hg than we had expected, potentially from enrolling wealthier households that may have an additional source of income. Compared with other birth cohorts, we have a smaller sample size that may limit our analyses.[4,96] Owing to limitations in the field, we relied on the Capurro somatic method to determine gestational age, which has been shown to overestimate gestational age for newborns <39 wk of age in Brazil; however, it can have high specificity (96%).[97–99]

The complexity of mineral and toxic metal transfer to the fetus during pregnancy makes evaluating both the maternal ME and the FE difficult, especially because of the inability to monitor nutrient status of the fetus *in utero*. The placenta is a vital organ that regulates nutrient and oxygen transfer to the fetus. Its impairment by toxic metals is yet another route through which toxic metals induce harmful outcomes.[100–102] Future research that incorporates the placenta and its ability to regulate minerals and toxic metal transfer may provide valuable insight on neonatal health. In addition, data that incorporates dietary, supplemental, and maternal toxic metal exposures throughout pregnancy, as well as ultrasound measurements and placenta characteristics, may provide important insight into the dynamic changes that occur during pregnancy.

SEMs also provide the framework to evaluate different groups to determine whether they fit the same data structure. Although this study did not have a sufficient sample size to conduct such analyses, evaluating how anemia status (yes/no) at term or toxic metal exposure status (high/low) may provide valuable insight on how perturbations of a single metal effect the rest of the covariance structure.

## Conclusions

When adjusting for the natural physiological hierarchy of mineral and toxic metal levels in mothers and newborns, our study supports that Pb exposures at levels below internationally recognized thresholds can impair newborn health. Although the study was designed to detect an effect of Hg on child developmental outcomes, Hg was not directly associated with birth weight or gestational age. This could have been because the study was powered to detect cognitive delays following *in utero* Hg exposure and did not incorporate adjustments for mixed exposures (and minerals) or birth outcomes specifically. We also find the number of prenatal care visits is a frequently overlooked aspect in environmental health studies that can be used to improve neonatal health. In addition, SEMs provide a robust statistical approach to evaluate risk factors for neonatal health that allows correlated end points to be analyzed jointly. Both minerals and toxic metals, alongside with antenatal care, and maternal characteristics are important determinants for neonatal health outcomes and need to be assessed jointly.

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The data sets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

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